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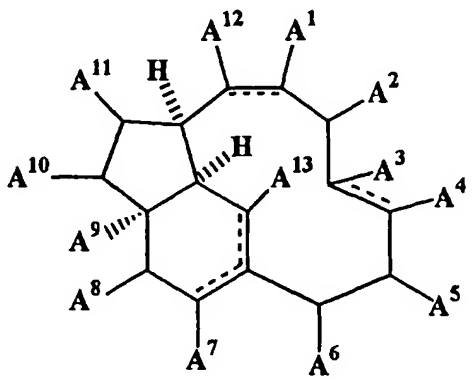
(54) Title: NOVEL ANTIBIOTIC COMPOUNDS



(1)

(57) Abstract: A method for treating a microbial infection or disease in a subject, said method comprising administering to said subject an effective amount of a compound according to the formula (1). Wherein; ---- denotes a single or double bond or an epoxidised bond, and A^1 to A^{13} are independently selected from moieties as depicted in the description. Also claimed are methods for disinfecting surfaces using the above compound, and claims to the above compound.

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NOVEL ANTIBIOTIC COMPOUNDS

Field of the Invention:

This invention relates to a novel class of antibiotic compounds and their use for treatment of various microbial infections and diseases in humans and other animals.

Background of the Invention:

Antibiotics, compounds with selective toxicity against infectious microorganisms, present humanity with enormous benefits and are credited with saving many millions of lives since their introduction in the 20th century. Today there is a continuing need for new antibiotics to assist in the management of multiply resistant pathogens (e.g. multiply resistant *Staphyloccus aureus* or vancomycin-resistant enterococcus) or to provide improved therapies for difficult-to-treat pathogens such as *Mycobacterium tuberculosis,* the causative agent of tuberculosis). Selectively toxic compounds also have utility as veterinary antibiotics and growth enhancers, where there is a need to develop agents with different modes of action from those used in humans, and also as preservatives and antisepsis agents in a wide range of medical and industrial processes and products.

Insects and terrestrial invertebrates face infection by many opportunistic microbial pathogens, yet they are a successful group of organisms which have been present on earth for hundreds of millions of years and are today represented by many millions of species, far more than any other group of macroorganisms. Insects and other terrestrial invertebrates must therefore have efficient methods for avoiding or overcoming potential infections.

Insects share with mammals and other organisms an "innate" immune system based on non-specific phagocytosis of foreign material by haemocytes, and production of a range of antimicrobial peptides such as defensins, cecropins and attacins in response to general microbial inducers such as lipopolysaccharide and (1,3)-beta-D-glucans. However, there has been no evidence from insects, or any other invertebrate, for the presence of a clonal, inducible-immune system of the B-lymphocyte/T-lymphocyte type that typifies mammalian responses to infection. Insects may therefore have other,

undiscovered, defensive systems to protect themselves against microbial invasion.

There has been little previous evidence for the synthesis of non-peptide antibiotics by insects. A survey of 102 species of North American arthropods in the 1950's (DeCoursey, Webster et al. 1953) revealed only two active extracts, and these were presumed to be active due to the presence of quinones, reactive compounds of no value as antibiotics. An antibacterial compound, *para*-hydroxycinnamaldehyde has recently been isolated from a Korean sawfly (Leem, Jeong et al. 1999), however no data on the mammalian toxicity of this compound was presented.

Between 1997 and 1999, the present applicants assembled a large collection of terrestrial invertebrates from the east coast of Australia and extracted a number of them and screened the extracts for biological activity. One particular extract from an Australian species of termite, *Nasutitermes triodiae* (Isoptera:Termitidae) (Froggatt), was shown to have antimicrobial activity against the Gram positive organism *Bacillus subtilis*. The extract was also shown to have only intermediate levels of growth-inhibitory activity against two transformed mammalian cells, namely SP2/O-Ag8 a non-secreting mouse myeloma cell line derived from Balb/C mice, and NCI-H460 a human-derived small cell lung carcinoma cell line.

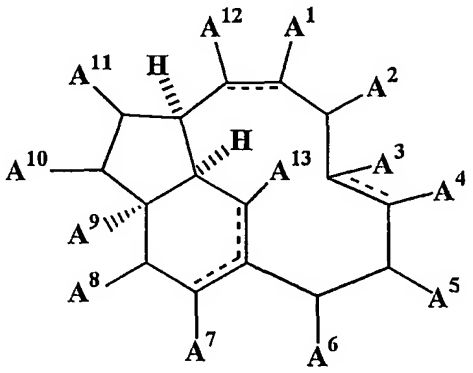
Four compounds have been purified to homogeneity from the extract of *N. triodiae*. These were a triol of a trinervitadiene (Formula (6)); a monoacetate of the same triol (Formula (8)); and two diols with the same trinervitadiene carbon skeleton (Formulae (7) and (9)). In addition, the triacetate (Formula (10)) of the aforementioned triol was synthesised by esterification with acetic anhydride. Of these compounds, all but one of the diols (9) represents a previously unreported structure. Furthermore, all of the compounds had measurable antimicrobial activity, a property not previously reported for any trinervitadiene. The triol (6) was shown to have moderately good antimicrobial potency against the target organism. The novel diol (7) had similar antimicrobial potency to the triol, while the known diol (9) was 2-4 times more potent. The monoacetate and triacetate were also both active, albeit less potent than the diols or triol. The triol (6) was also tested in mammalian cell culture and shown to have selective toxicity for the test microorganism over mammalian cells.

These trinervitadiene compounds therefore have potential utility as human or veterinary antibiotics or as antiseptic agents in industrial or other processes. Furthermore, because the results provided herein demonstrate for the first time that derivatives of the trinervitadiene carbon skeleton have antimicrobial properties, it may reasonably be concluded that other derivatives of this carbon skeleton will also have similar selective antimicrobial properties.

Disclosure of the Invention:

Thus, in a first aspect, the present invention provides a method for treating a microbial infection or disease in a subject, said method comprising administering to said subject an effective amount of a compound according to the formula:

(1)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

(i) substituents A^1 to A^{13} are selected, independently, from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy, or

(ii) any one or more of substituent pairs A^1 and A^2, A^1 and A^3, A^2 and A^3, A^2 and A^4, A^3 and A^4, A^3 and A^5, A^4 and A^5, A^4 and A^6, A^5 and A^6, A^6 and A^7, A^7 and A^8, A^7 and A^9, A^8 and A^9, A^8 and A^{10}, A^9 and A^{10}, A^9 and A^{11}, A^{10} and A^{11}, A^{11} and A^{12}, A^1 and A^{12}, and A^2 and A^{12} form a substituted or

unsubstituted heterocyclic group, wherein any substituents, including A^{13}, not forming a substituted or unsubstituted heterocyclic ring, are selected independently from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy;

with the provisos that,

only one of the bonds between C1 and C2, and C1 and C15, may be a double bond or epoxide,

when the bond between C1 and C2 is a double bond or epoxide, A^7 is bound to C2 by a single bond,

when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,

when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and

when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;

and pharmaceutically/veterinary-acceptable salts thereof.

The term "lower" is intended to mean a group having 1 to 6 carbon atom(s), unless otherwise provided.

Suitable "lower alkyl" and lower alkyl moieties in the terms "lower alkoxy", "lower alkythio", "lower alkylamino", "lower alkylsulfonyl", "lower alkylsulfinyl" and "lower alkylsulfonyloxy" may be straight or branched such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl or the like.

Suitable "lower alkene" groups may be CH_2, $CHCH_3$, $CHCH_2$, $CHCHCH_3$ and the like. Similarly, suitable "lower alkyne" groups may be CH, CCH_3, CCH, $CCCH_3$ and the like.

Suitable "lower alkoxy" may be methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, tert-butoxy and the like.

Suitable "lower carboxy" may be carboxymethyl, carboxyethyl, carboxypropyl, carboxyisopropyl,carboxybutyl, carboxyisobutyl, carboxy tert-butyl and the like.

A suitable "lower aldehyde group" may be selected from aldehyde groups such as methanal, ethanal, propanal, isopropanal , butanal, isobutanal, tert-butanal and the like.

A suitable "lower ketone group" may be selected from ketone groups such as methanone, ethanone, propanone and the like.

A suitable "lower ester group" may be methanoate, ethanoate, propanoate, isopropanoate, butanoate, isobutanoate, tert-butanoate and the like.

A suitable "lower acyloxy group" may be acetoxy, propionyloxy, butyryloxy and the like.

A suitable "lower alcohol group" may be methanol, ethanol, propanol, isopropanol, butanol, isobutanol, tert-butanol and the like.

Suitable "lower alkylthio" include methylthio, ethylthio, propylthio, butylthio and the like, and lower alkyl thio substituted lower alkyl such as methylthiomethyl, methylthioethyl, methylthiopropyl, methylthiobutyl, ethylthiomethyl, ethylthioethyl, ethylthiopropyl, ethylthiobutyl and the like.

Suitable "lower alkylamino" include methylamino, ethylamino, propylamino, butylamino and the like, and mono or di(lower alkyl) amino substituted lower alkyl such as methylaminomethyl, methylaminoethyl, methylaminopropyl, methylaminobutyl, ethylaminomethyl, ethylaminoethyl, ethylaminopropyl, ethylaminobutyl, dimethylaminomethyl, dimethylaminoethyl, dimethylaminopropyl, dimethylaminobutyl, diethylaminomethyl, diethylaminoethyl, diethylaminopropyl, diethylaminobutyl and the like.

Suitable "lower alkylsulfonyl" may be methylsulfonyl, ethylsulfonyl, propylsulfonyl, butylsulfonyl and the like.

Suitable "lower alkylsulfinyl" include methylsulfinyl, ethylsulfinyl, propylsulfinyl, butylsulfinyl and the like.

Suitable "lower alkylsulfonyloxy" include methylsulfonyloxy , ethylsulfonyloxy, propylsulfonyloxy, butylsulfonyloxy and the like.

Suitable substituted or unsubstituted heterocyclic groups may be groups having a carbon and oxygen backbone of 5 to 8 atoms (inclusive of the 2-3 carbon atoms contributed by the Formula (**1**) structure), including cyclic acetals and cyclic carbonates. Such heterocyclic groups may be substituted by one or more of OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups,

lower ester groups, lower acyloxy, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy.

Preferably, A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, O, SH, NH_2 and OR. More preferably, A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH and OR. R in the group OR is a lower alkyl as defined above (preferably, methyl or ethyl) or lower acyl.

Preferably, A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy and lower alcohol groups. More preferably, A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, and A^9 is selected from methanol and CH_2OR groups. Again, R in the group OR is a lower alkyl as defined above (preferably, methyl or ethyl) or lower acyl.

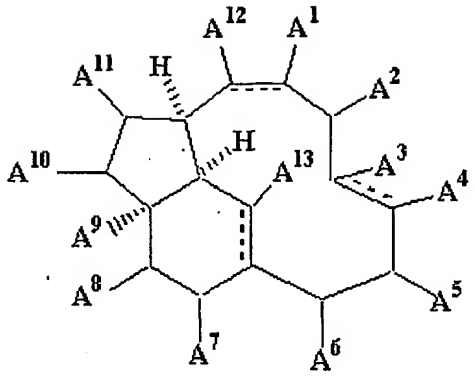
Preferably, A^{12} is selected from lower alkyl, lower alkene or lower alkyne. More preferably, A^{12} is selected from methyl and CH_2. Most preferably, A^{12} is CH_2.

It is also preferred that at least two of said A^1 to A^{13} consist or comprise OH or OR groups, wherein R is as defined above.

Suitable pharmaceutically/veterinary-acceptable salts of the compound of formula (**1**) include non-toxic salts such as acid addition salts such as aninorganic acid addition salt (e.g. hydrochloride, sulfate, phosphate, etc.), an organic acid addition salt (e.g. formate, acetate, trifluoroacetate, etc.), a salt with an amino acid (e.g. arginine salt, etc.), a metal salt such as an alkali metal salt (e.g. sodium salt, potassium salt, etc.) and an alkaline earth metal salt (e.g. calcium salt, magnesium salt, etc.), an ammonium salt, an organic base addition salt (e.g. trimethylamine salt, triethylamine salt, etc.) and the like.

Preferably, the compound used in the method of the present invention is of the formula:

(2)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, lower alkyl, lower alkene, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy and lower alcohol groups;

with the provisos that,

when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,

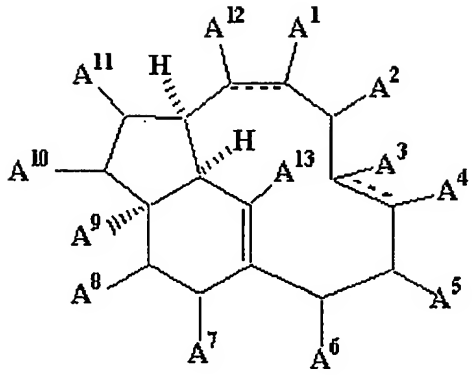
when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and

when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;

and pharmaceutically/veterinary-acceptable salts thereof.

More preferably, the compound used in the method of the present invention is of the formula:

(3)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, acetoxy, propionyloxy and butyryloxy groups, and methanol, ethanol, propanol and butanol groups;

with the provisos that,

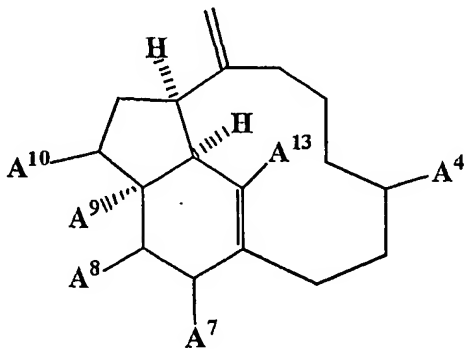
when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and

when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;

and pharmaceutically/veterinary-acceptable salts thereof.

Even more preferably, the compound used in the method of the present invention is of the formula:

(4)



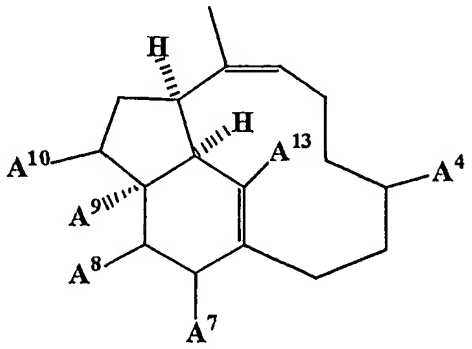
wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined above in relation to formula (1),

and pharmaceutically/veterinary-acceptable salts thereof;

or:

(5)



wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined above in relation to formula (1),

and pharmaceutically/veterinary-acceptable salts thereof.

Most preferably, the compound used in the method of the present invention is selected from;

1(15),8(19)-Trinervitadiene-3α,5α,18-triol,

1(15),8(19)-Trinervitadiene-3α,5α-diol,

1(15),8(19)-Trinervitadiene-3α,5α,18-triol 5-acetate,

1(15),8(9)-Trinervitadiene-2β,3α-diol, and

1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate.

For pharmaceutical and/or veterinary applications, the compound or pharmaceutically/veterinary-acceptable salt thereof, is formulated for administration by any of the commonly used routes such as oral, nasal, rectal, vaginal, intramuscular, intraveneous administration routes. For convenience, it is preferred that the compound is formulated for oral administration, wherein the compound or pharmaceutically/veterinary-acceptable salt thereof may be in admixture with commonly known binding materials and

excipients. Suitable oral formulations may be in the form of capsules, tablets, caplets or syrups.

Typically, the compound or pharmaceutically/veterinary-acceptable salt thereof, will be administered at an effective antimicrobial amount, such as 1 to 100 mg/kg, preferably 5 to 20 mg/kg.

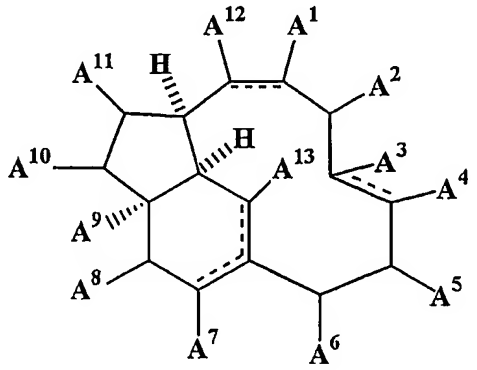
The method of the invention may be for the treatment of an antimicrobial infection or disease selected from, for example, bacterial infection of wounds including surgical wounds, lung infections (e.g. tuberculosis), skin infections, and systemic bacterial infections.

In a second aspect, the present invention provides a pharmaceutical and/or veterinary formulation for treating a microbial infection or disease in a subject, said formulation comprising a compound according to any of the formulae (1) to (5) in admixture with a suitable pharmaceutically/veterinary-acceptable excipient.

The compound of any of the formulae (1) to (5) may also be useful for other non-pharmaceutical/veterinary uses such as in disinfectants and cleaners.

Thus, in a third aspect, the present invention provides a method for disinfecting a surface (e.g. a hard surface such as kitchen bench tops, bathroom tiles and the like), said method comprising applying to said surface an amount of a compound according to the formula:

(1)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

(i) substituents A^1 to A^{13} are selected, independently, from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy, or

(ii) any one or more of substituent pairs A^1 and A^2, A^1 and A^3, A^2 and A^3, A^2 and A^4, A^3 and A^4, A^3 and A^5, A^4 and A^5, A^4 and A^6, A^5 and A^6, A^6 and A^7, A^7 and A^8, A^7 and A^9, A^8 and A^9, A^8 and A^{10}, A^9 and A^{10}, A^9 and A^{11}, A^{10} and A^{11}, A^{11} and A^{12}, A^1 and A^{12}, and A^2 and A^{12} form a substituted or unsubstituted heterocyclic group, wherein any substituents, including A^{13}, not forming a substituted or unsubstituted heterocyclic ring, are selected independently from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy;

with the provisos that,

only one of the bonds between C1 and C2, and C1 and C15, may be a double bond or epoxide,

when the bond between C1 and C2 is a double bond or epoxide, A^7 is bound to C2 by a single bond,

when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,

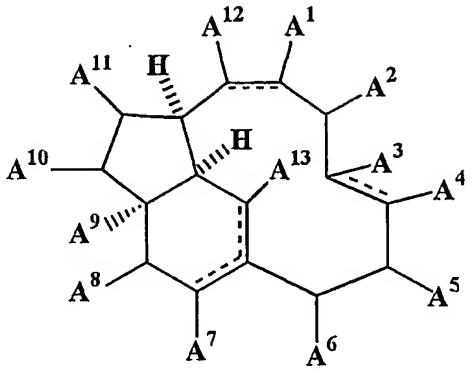
when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and

when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;

and salts thereof.

In a fourth aspect, the present invention provides an antimicrobial compound of the formula:

(1)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

(i) substituents A^1 to A^{13} are selected, independently, from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy, or

(ii) any one or more of substituent pairs A^1 and A^2, A^1 and A^3, A^2 and A^3, A^2 and A^4, A^3 and A^4, A^3 and A^5, A^4 and A^5, A^4 and A^6, A^5 and A^6, A^6 and A^7, A^7 and A^8, A^7 and A^9, A^8 and A^9, A^8 and A^{10}, A^9 and A^{10}, A^9 and A^{11}, A^{10} and A^{11}, A^{11} and A^{12}, A^1 and A^{12}, and A^2 and A^{12} form a substituted or unsubstituted heterocyclic group, wherein any substituents, including A^{13}, not forming a substituted or unsubstituted heterocyclic ring, are selected independently from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy;

with the provisos that,

only one of the bonds between C1 and C2, and C1 and C15, may be a double bond or epoxide,

when the bond between C1 and C2 is a double bond or epoxide, A^7 is bound to C2 by a single bond,

when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,
when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and
when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;
and salts thereof, with the further proviso that said compound is not 1(15),8(9)-Trinervitadiene-2β,3α-diol.

Preferably, the compound of the fourth aspect is in a substantially purified form.

In a fifth aspect, the present invention provides an antimicrobial trinervitadiene compound in a substantially purified form, said compound being obtainable from a termite of the genus Nasutitermes.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

The invention will hereinafter be further described by reference to the following non-limiting examples.

Example 1:

METHODS AND MATERIALS

A sample comprising 11.59 g wet weight of *Nasutitermes triodiae* (Isoptera:Termitidae) (Froggatt) adults (mixed castes; mainly soldiers) was collected manually in the field and the sample was snap frozen in a dry-shipper containing liquid nitrogen. The specimen was stored at -80°C before being freeze-dried to constant weight (1.39g). The sample was ground to a powder and dispersed in 49 mL of 70% (v/v) methanol in water and shaken at

room temperature overnight. The sample was filtered and centrifuged and the supernatant was recovered. The combined residues were then re-extracted with a further 20 mL of 70% methanol. The supernatants were combined to a total of 47 mL.

Antimicrobial activity was detected by saturating a 1/4 inch diameter filter paper disk (Bacto) with the methanolic extract, evaporating the solvent in a cool air stream and placing the disk onto a bacteriological plate containing *Bacillus subtilis* (ATCC strain 6633; 9.2 mL of a log phase culture with Abs_{600nm} = 1 per 200 mL of Luria-Bertani medium containing 1.5% (w/v) agar). The plate was incubated at 28°C for 24 hours and the diameter of the clearing zone was measured. Dilutions of the extract and fractions from HPLC chromatography were tested in the same way. In some cases, fractions from the column were tested by evaporating them to dryness, redissolving in methanol and simply spotting 10μL of the methanolic sample directly onto the bacteriological plate and proceeding as described.

For the purification of the compound of fraction 23, (6) 6 mL of the methanolic extract was purified in 12 x 0.5 mL batches by semi-preparative reverse-phase HPLC over a YMC ODS-AQ capped C18 column (250 mm x 10 mm) (Sapphire Biosystems) under the following conditions:

Loading conditions were:	0.5 mL of extract for each batch Solvent A = 99.95% water + 0.05% (v/v) trifluoroacetic acid Solvent B = 100% acetonitrile
Elution conditions were:	0-2 minutes 100% A 2-22 minutes linear gradient 0-100% B 22-35 minutes 100% B flow rate 4 mL/minute

Fractions were collected by time (1 minute per fraction). The absorbance of the effluent was monitored at 230nm.

Corresponding fractions were pooled across all 12 batches and the eluate in each of the pooled fractions was evaporated to dryness under nitrogen. The residues were weighed and taken up again in small volumes of appropriate solvents - methanol for preparative or analytical HPLC and

electrospray mass spectroscopy, deuterated chloroform for nuclear magnetic resonance (nmr) spectroscopy, etc.

For compounds in fractions other than fraction 23, a similar protocol was used but with the following additional steps. An extra 4 mL of extract was used and the eluates from all 20 batches were pooled and processed as described above. The material in fractions 24 and 26 was a mixture after this first preparative HPLC step, therefore the pooled active fractions were further purified using one of the two following isocratic chromatographic procedures.

In both isocratic purifications the same YMC ODS-AQ capped C18 column (250 mm x 10 mm) (Sapphire Biosystems) was used as in the first step.

Isocratic procedure 1
(used to purify fraction 24, (7) in 4 batches)

Loading conditions were:	for each batch, 0.5 mL of fraction 24 (7) in methanol
Elution conditions were:	Acetonitrile: tetrahydrofuran: water* = 42:28:30 for 20 minutes

Isocratic procedure 2
(used to purify fraction 26, (**8** and **9**) in 2 batches)

Loading conditions were:	for each batch 0.5 mL of fraction 26 (**8** and **9**) in methanol
Elution conditions were:	acetonitrile : water* = 80 : 20 for 25 minutes

(*water contained 0.05% trifluoroacetic acid (v/v))

The purified fractions were examined by analytical HPLC using similar gradient elution conditions to the preparative procedure, i.e. a water–acetonitrile gradient, followed by 100% acetonitrile. The only differences were that the analytical column was a YMC ODS-AQ capped C18 column

(250 mm x 3 mm), the flow rate was 0.55 mL/minute and 20μL of sample was loaded onto the column for each run.

The purity and composition of the active pooled fractions were determined using a range of standard spectroscopic techniques including electrospray mass spectroscopy (ESMS), high resolution electron impact mass spectroscopy (HREIMS), electron impact mass spectroscopy (EIMS) and 300 and 500 MHz proton and carbon nuclear magnetic resonance in one and two dimensional modes.

Effects on mammalian cell growth were determined by exposing cultures of two mammalian neoplastic cell lines (SP2/0-Ag8, a non-secreting mouse myeloma cell line derived from Balb/C mice, and NCI-H460, a human-derived small cell lung carcinoma line) to fixed dilutions of the methanolic extract of *N. triodiae* or to fixed concentrations of fraction 23, (6) for 19 hours at 37°C. Cells were grown in wells of sterile 96-well tissue culture cluster plates by standard methods. Cell growth was estimated using the Cell Proliferation Reagent WST-1 (Roche Diagnostics) according to the manufacturer's instructions and proliferation data were compared with those from untreated control wells.

Minimum inhibitory concentrations with *Bacillus subtilis* ATCC strain 6633 were determined using the National Committee for Clinical Laboratory Standards broth microdilution test (NCCLS, 2000. NCCLS Document M7-A5 - Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard - Fifth Edition). The test was standardised using penicillin G and gentamicin with *Staphyloccous aureus* ATCC strains 29213 and 25923 and *Enterococcus faecalis* ATCC strain 29212. Results of standardisation were assessed according to the NCCLS standards (NCCLS, 2000. NCCLS Document M100-S10(M7) - Performance Standards for Antimicrobial Susceptibility Testing; Tenth Informational Supplement (Aerobic Dilution). NCCLS, Wayne, Pennsylvania).

RESULTS

The crude 70% methanol extract of *N. triodiae* displayed antimicrobial activity against *B. subtilis* (clear zone diameter 9 mm in the standard filter disk test) and moderate inhibitory activity against mammalian cells (37% of control at a concentration of approximately 30 μg/mL). There was no activity against a test strain (ACM 3221) of *Escherichia coli* (a Gram negative bacterium) in a similar test protocol.

After chromatographic fractionation of the methanolic activity, antimicrobial activity was detected in the fraction eluting between 22 and 23 minutes (fraction 23) and also the fractions eluting between 23 and 24 minutes (fraction 24) and between 25 and 26 minutes (fraction 26).

Fraction 23

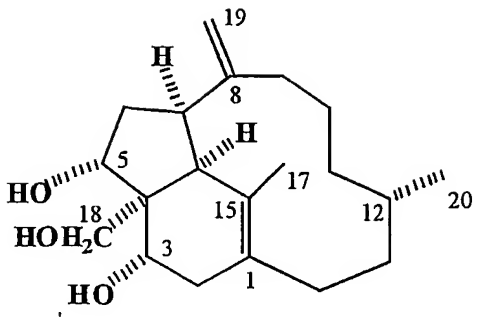
A total of 9 mg of the pure compound was purified from 6 mL of crude extract, indicating a starting concentration of 1.5 mg/mL.

The molecular formula of the compound in fraction 23 (**6**) was determined as $C_{20}H_{32}O_3$ by ESMS which show sodiated ions at m/z 343 (MNa^+) and m/z 663 (M_2Na^+) and by HREIMS which showed M^+-H_2O at m/z 302.2243 where m/z calculated for $C_{20}H_{30}O_2$ is 302.2246.

The ^1H NMR and ^{13}C NMR chemical shift data for the compound (**6**) in fraction 23 are shown in Table 1. The compound in fraction 23 was determined to be 1(15),8(19)-trinervitadiene-3α,5α,18-triol (**6**). This compound has not been reported previously.

The minimum inhibitory concentration of compound (**6**) against *B. subtilis* was estimated as ≤50 μg/mL. Purified compound (**6**) had no detectable inhibitory effect on the proliferation of NCI-H460 cells at concentrations up to 100 μg/mL. Compound (**6**) had no detectable inhibitory effect on the proliferation of SP2/0 cells at concentrations up to 30 μg/mL.

(6)



1(15),8(19)-Trinervitadiene-3α,5α,18-triol

Fraction 24

The material in fraction 24, which also showed antimicrobial activity, was a mixture of at least two compounds after the first chromatographic step. It was therefore submitted to "Isocratic procedure 1" as described above and the single biologically active u.v.-absorbing peak which eluted between 14 and 17 minutes was collected.

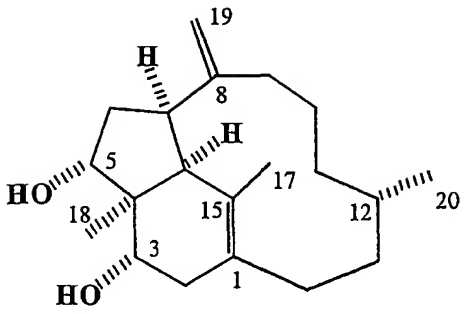
A total of 4 mg of the pure biologically active compound present in fraction 24 was purified from 10 mL of starting material indicating an approximate starting concentration of 0.4 mg/mL in the crude extract.

The molecular formula of the compound in fraction 24 (7) was determined as $C_{20}H_{32}O_2$ by ESMS which show sodiated ions at m/z 327 (MNa^+) and by HREIMS which showed M^+ at m/z 304.2403 where m/z calculated for $C_{20}H_{32}O_2$ is 304.2402.

The ^1H NMR and ^{13}C NMR chemical shift data for the compound (7) in fraction 24 are shown in Table 2. The compound in fraction 24 was determined to be 1(15),8(19)-trinervitadiene-3α,5α-diol (7). This compound has not been reported previously.

5 μg of compound (7) gave a clear zone of diameter 7 mm in the disc diffusion assay. The minimum inhibitory concentration of compound (7) against *B. subtilis* was estimated as ≤50 μg/mL.

(7)



1(15),8(19)-Trinervitadiene-3α,5α-diol

Fraction 26

The material in fraction 26 was a mixture of at least two biologically active compounds after the first chromatographic step. It was therefore submitted to "Isocratic procedure 2" as described above and two active fractions were collected. The first, which eluted from the column at approximately 12 minutes is designated Fraction 26A, the second, which eluted from the column at approximately 14 minutes is designated Fraction 26B.

Fraction 26A

A total of 0.5 mg of the pure biologically active compound present in fraction 26A was purified from 10mL of starting material indicating an approximate starting concentration of 0.05 mg/mL in the crude extract.

The molecular formula of the compound in fraction 26A (**8**) was determined as $C_{22}H_{34}O_4$ by ESMS which showed ions at *m/z* 345 (MH^+-H_2O) 363 (MH^+), 385 (MNa^+), 747 (M_2Na^+) and by HREIMS which showed M^+ at m/z 362.2460, where $C_{22}H_{34}O_4$ requires 362.2457; and M^+-H_2O at *m/z* 344.2350 where $C_{22}H_{32}O_3$ requires 344.2351.

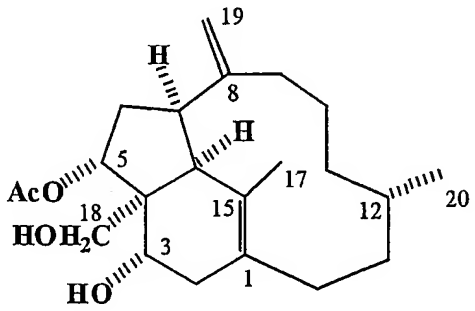
The ^1H NMR chemical shift data for the triol monoacetate (**8**) in fraction 26A are shown in Table 3. The identity of the triol monoacetate was further confirmed by partially acetylating the triol (**6**) and confirming the presence in the acetylation mixture of a major component with identical retention time and ^1H NMR spectrum to the natural triol monoacetate. The protocol used was as follows:

The triol (6) (1 mg) and acetic anhydride (10 μL) in dry pyridine (100 μL) were kept for 3 hours at room temperature. The reaction was diluted with water, extracted with dichloromethane, and the extract subjected to preparative HPLC on the standard column using gradient elution in acetonitrile/water from 50:50 to 100:0. The fraction with retention time 18 min when analysed under normal gradient elution conditions contained a major component with identical retention time to the natural triol monoacetate (8). The ^{1}H NMR spectrum of this major component (Table 3) also matched that of the natural triol monoacetate (8).

The compound in fraction 26A was therefore determined to be 1(15),8(19)-trinervitadiene-3α,5α,18-triol 5-acetate (8). This compound has not been reported previously.

An unknown concentration and mass of the compound (8) gave a clear zone of diameter 15 mm in the disc diffusion assay. The minimum inhibitory concentration of compound (8) in the standard broth microdilution assay against *B. subtilis* was estimated as >50μg/mL.

(8)



1(15),8(19)-Trinervitadiene-3α,5α,18-triol 5-acetate

Fraction 26B

A total of 1.5 mg of the pure biologically active compound present in fraction 26B was purified from 10 mL of starting material indicating an approximate starting concentration of 0.15 mg/mL in the crude extract.

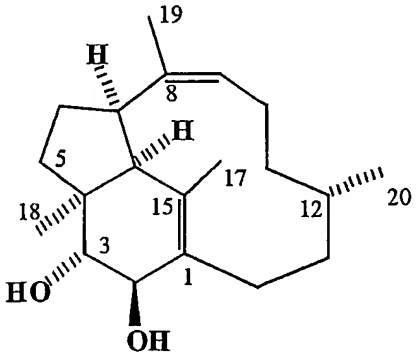
The molecular formula of the compound in fraction 26B (9) was determined as $C_{20}H_{32}O_2$ by ESMS which showed sodiated ions at *m/z* 327

(MNa^+), 631 (M_2Na^+) and by HREIMS which showed M^+ at *m/z* 304.2404 where $C_{20}H_{32}O_2$ requires 304.2402.

The ^1H NMR chemical shift data for the trinervitadiene diol (**9**) in fraction 26B are shown in Table 4. The compound in fraction 26B was therefore determined to be 1(15),8(9)-trinervitadiene-2β,3α–diol (**9**) by comparison of the measured chemical shifts with previously published ^1H NMR data (Goh, Chuah *et al.*, 1984; Braekman, Daloze *et al.*, 1983; Prestwich & Collins, 1981; Prestwich *et al.*, 1976b)for this compound (Table 4).

An unknown concentration and mass of the compound (**9**) gave a clear zone of diameter 15 mm in the disc diffusion assay. The minimum inhibitory concentration of compound (**9**) in the standard broth microdilution assay against *B. subtilis* was estimated as ≤ 25 μg/mL. Although the structure of this compound has been published previously, it has not previously (e.g. Goh, Chuah *et al.*, 1984; Braekman, Daloze *et al.*, 1983; Prestwich & Collins, 1981; Prestwich *et al.*, 1976b) been reported that it has potent antimicrobial activity.

(**9**)



1(15),8(9)-Trinervitadiene-2β,3α–diol

1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate (10)

1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate (**10**) was synthesised by acetylation of the triol (**6**). The triol (**6**) (1 mg) and acetic anhydride (30 μL) in dry pyridine (100 μL) were kept for 3 days at room temperature. The reaction was diluted with water, extracted with dichloromethane, and the extract subjected to preparative HPLC under the

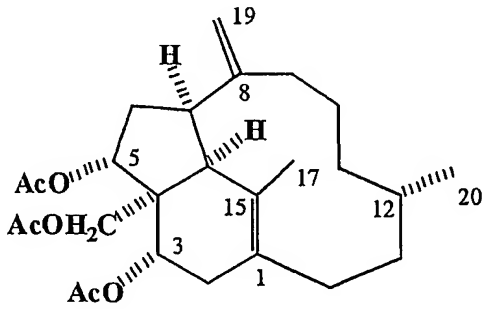
standard conditions. The major fraction was collected and filtered through a short silica column in dichloromethane to afford the triacetate (**10**) (1 mg), which eluted as a single major peak at 28 minutes when chromatographed under the standard analytical HPLC conditions described in the above Materials and Methods section.

The molecular formula of the reaction product was confirmed as $C_{26}H_{38}O_6$ by ESMS which showed sodiated ions at *m/z* 469 (MNa^+) and by HREIMS which showed (M^+-AcOH) at *m/z* 386.2449 where $C_{24}H_{34}O_4$ requires 386.2457.

The ^1H NMR chemical shift data for the trinervitadiene triol triacetate (**10**) reaction product are shown in Table 5. The acetylation product was therefore determined to be 1(15),8(19)-trinervitadiene-3α,5α,18-triol 3,5,18-triacetate (**10**). This compound has not been reported previously.

5 μg of compound (**10**) gave a clear zone of diameter 11 mm in the disc diffusion assay. The minimum inhibitory concentration of compound (**10**) against *B. subtilis* was estimated as >50μg/mL.

(**10**)



1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate

Example 2:

In studies on the antimicrobial activity of other Australian termites of the genus Nasutitermes (Family: Termitidae; Sub-Family: Nasutitermitinae) it was noted that extracts of three other species, *Nasutitermes exitiosus* (Hill) and two unidentified species of the same genus, exhibited antimicrobial activity which eluted at the same retention time as 1(15),8(9)-Trinervitadiene-2β,3α–diol (**9**) and shared identical ^1H NMR chemical shift data (Table 4) with the diol (**9**) purified from fraction 26B.

It was also noted that extracts made exclusively from workers of the species *N. exitiosus* exhibited no antimicrobial activity and did not exhibit any of the characteristic u.v. absorbing peaks attributed to trinervitadiene derivatives, which elute in the 22-28 minute region of the standard gradient HPLC chromatogram. On the other hand, an extract of soldiers from the same nest as the aforementioned workers exhibited antimicrobial activity.

When the extract of soldier termites was subjected to the standard gradient HPLC chromatography, the biologically active u.v.-absorbing peaks characteristic of trinervitadiene derivatives were observed. This indicates that separation of soldier termites from workers prior to their extraction may improve the efficiency both of detecting and purifying biologically active trinervitadiene derivatives.

Discussion:

A range of compounds with the trinervitadiene carbon skeleton have previously been reported from termites (for example: Prestwich, Tanis et al. 1976a,b; Vrkoc, Budesinsky et al. 1978a,b; Dupont, Braekman et al. 1981; Prestwich, Spanton et al, 1981; Baker & Walmsley, 1982; Braekman, Daloze et al. 1986). Trinervitadiene derivatives have been found in extracts of a number of species of termites belonging to nine genera of termites within the subfamily Nasutitermitinae of the family Termitidae. However, this is the first time that the isolation of compounds (**6, 7, and 8**) have been reported or that the triacetate(**10**) of compound (**6**) has been prepared. The novelty of these compounds is underlined by the fact that no trinervitadiene derivatives have previously been reported with hydroxylation or other substitutions at positions 5 and 18 on the carbon skeleton (e.g. compound (**6**)). Furthermore, no data have been reported previously regarding antimicrobial activity of any trinervitadiene or derivative thereof including the compounds whose isolation or preparation is described herein (**6, 7, 8, 9** and **10**).

It has been determined that compound (**6**) has a minimum inhibitory concentration in the range ≤ 50 parts per million and that it is at least twice as toxic to microbial cells as it is to human cells and potentially significantly more selective than this. Compound (**9**) has a minimum inhibitory concentration in the range of ≤ 25parts per million and has significant inhibitory activity as low as 12 parts per million. Compounds (**7**), (**8**) and (**10**) have reduced but detectable antimicrobial activity. For example, acetylation of the hydroxyl groups seems to reduce but not abolish activity, whilst it is also clear that the number and arrangement of groups on the trinervitadiene carbon skeleton modulates the level of antimicrobial activity.

Compounds (**6, 7, 8, 9** and **10**) and a range of other trinervitadiene derivatives where the number, position and nature of groups is varied (such as the specified derivatives of compounds **11-16**) can therefore be expected to have utility as antibiotics or for some of the other purposes mentioned above. Alternatively, they may be useful lead compounds for the development of derivatives with enhanced antibiotic activities

It is interesting that prolonged investigations of the role of soldier defensive secretions has led to the classification of the trinervitadiene derivatives as "defensive compounds" (i.e. the assumption has been that their

function is solely in defence of the termite colony against attack by invertebrate or vertebrate predators). However, the present discovery that these compounds have antimicrobial activity raises the possibility that they also function naturally to suppress microbial parasites within the termite colony.

Table 1: 1(15),8(19)-Trinervitadiene-3α,5α,18-triol (6)
^1H and ^{13}C NMR data ($CDCl_3$):

Position	δ C[a]	δ H[b,c]
1	127.9	-
2	36.6	2.40 (m), 2.18 (m)
3	70.1	4.16 (bs)
4	53.3	-
5	74.8	4.68 (d, 3.5)
6	36.3	2.18 (m), 1.84 (m)
7	49.2	3.47 (m)
8	149.7	-
9	27.2	1.92 (m)1.83 (m),
10	23.8	1.59 (m), 1.59 (m)
11	31.9	1.21 (m), 0.91 (m)
12	27.3	1.31 (m)
13	32.0	1.43 (m), 1.43 (m)
14	27.8	2.40 (m), 1.68 (m)
15	126.4	-
16	51.9	2.55 (d, 11.5)
17	21.0	1.68 (s)
18	64.2	3.95 (d, 11.0), 3.80 (bs)
19	113.3	4.99 (s), 4.86 (s)
20	21.8	0.88 (d, 7.0)

[a] Chemical shifts, (δ) with $CDCl_3$ (76.9) as reference for solutions in $CDCl_3$ at 75.43 MHz.

[b] Chemical shifts (δ) with $CHCl_3$ (7.26) as reference for solutions in $CDCl_3$ at 500 Mhz.

[c] Where individual proton multiplets are unresolved chemical shifts are approximate.

Table 2: 1(15),8(19)-Trinervitadiene-3α,5α-diol (7)

^{1}H and ^{13}C NMR data ($CDCl_3$):

Position	δ C	δ H
1	127.7	-
2	36.8	2.29 (dd, 6, 16.5) 1.95 (m)
3	68.7	3.97 (dd, 11.0, 6.5)
4	49.9	-
5	76.9	4.27 (d, 4)
6	36.6	2.16 (m), 1.75 (m)
7	49.0	3.46 (m)
8	150.2	-
9	27.0	1.98 (m), 1.80 (m)
10	23.9	1.58 (m), 1.58 (m)
11	32.0	1.20 (m), 0.93 (m)
12	27.2	1.36 (m)
13	32.1	1.36 (m), 1.44 (m)
14	27.9	2.41 (m), 1.70 (m)
15	126.7	-
16	55.6	2.70 (d, 10.5)
17	21.2	1.71 (s)
18	12.4	0.98 (s)
19	112.9	4.98 (s), 4.85 (s)
20	21.8	0.89 (d, 6.5)

Table 3: 1(15),8(19)-Trinervitadiene-3α,5α,18-triol 5-acetate (8)
^{1}H NMR data ($CDCl_3$):

Position	Natural acetate δ H	Acetylation product δ H
H-3	4.14 (m)	4.14 (m)
H-5	5.72 (d, 4.0)	5.72 (d, 4.5)
H-7	3.40 (m)	3.40 (m)
H-17	1.67 (s)	1.67 (s)
H-18	3.85 (d, 13.0) 3.40 (d, 13.0)	3.85 (d, 13.0) 3.40 (m)
H-19	5.02 (d, 2.0) 4.90 (d, 2.0)	5.02 (d, 2.0) 4.90 (d, 2.0)
H-20	0.88 (d, 6.0)	0.89
$OCOCH_3$	2.18 (s)	2.17 (s)

Table 4: 1(15),8(9)-Trinervitadiene-2β,3α–diol (9)

^{1}H NMR data ($CDCl_3$):

Position	This patent δH	Goh, Chuah *et al.* (1984) ($CDCl_3$)	Braekman, Daloze *et al.* (1983) ($CDCl_3$)	Prestwich & Collins (1981) ($CDCl_3$)	Prestwich *et al.* (1976b)	Group
H-2	4.03 (d, 8)	4.0 (d)	4.05 (d, 10)	4.05 (br, d, 8)		CHOH
H-3	3.71 (d, 9)	3.76 (d)	3.70 (d, 10)	3.70 (d, 8.5)		CHOH
H-9	5.29 (dd 11.0, 6.0)		5.30 (dd, 10, 5)	5.30 (br, m)	5.28 (ddq, 12, 6, 1.8)	CH=
H-17	1.69 (br)	1.67 (d)	1.68 (bs)	1.69 (d, 0.6)		CH_3
H-18	0.97 (s)	0.97 (s)	0.95 (s)	0.99 (s)		CH_3
H-19	1.56 (d, 2.0)	1.49 (s)	1.58 (d)	1.57 (d, 1.2)	1.59 (d, 1.8)	CH_3
H-20	0.85 (d, 7.0)	0.90 (d, 6.6)	0.85 (d, 6)	0.86 (d, 6.1)		CH_3

Table 5: 1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate (10)
^{1}H NMR data ($CDCl_3$):

Position	δ H
3	5.36 (dd, 11.0, 6.5)
5	5.22 (d, 4.0)
7	3.43 (m)
16	2.90 (d, 12.0))
17	1.71 (s)
18	4.54 (d, 11.5) 4.04 (d, 11.5)
19	5.02 (s), 4.92 (s)
20	0.89 (d, 6.5)
$OCOCH_3$	2.09 (s) 2.06 (s) 1.98 (s)

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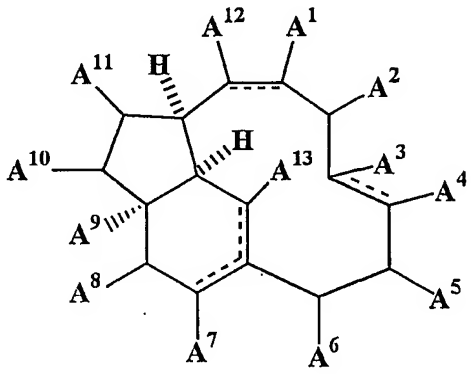
Vrkoc, J., M. Budesinsky, et al. (1978b). "Structure of trinervitene diterpenoids from *Nasutitermes rippertii* (Rambur)." Collection of Czechoslovak Chemical Communications 43: 1125-1132.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Claims:

1. A method for treating a microbial infection or disease in a subject, said method comprising administering to said subject an effective amount of a compound according to the formula:

(1)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

(i) substituents A^1 to A^{13} are selected, independently, from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy, or

(ii) any one or more of substituent pairs A^1 and A^2, A^1 and A^3, A^2 and A^3, A^2 and A^4, A^3 and A^4, A^3 and A^5, A^4 and A^5, A^4 and A^6, A^5 and A^6, A^6 and A^7, A^7 and A^8, A^7 and A^9, A^8 and A^9, A^8 and A^{10}, A^9 and A^{10}, A^9 and A^{11}, A^{10} and A^{11}, A^{11} and A^{12}, A^1 and A^{12}, and A^2 and A^{12} form a substituted or unsubstituted heterocyclic group, wherein any substituents, including A^{13}, not forming a substituted or unsubstituted heterocyclic ring, are selected independently from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy;

with the provisos that,
only one of the bonds between C1 and C2, and C1 and C15, may be a double bond or epoxide,
when the bond between C1 and C2 is a double bond or epoxide, A^7 is bound to C2 by a single bond,
when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,
when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and
when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;
and pharmaceutically/veterinary-acceptable salts thereof.

2. The method of claim 1, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, O, SH, NH_2 and OR, and R in the group OR is a lower alkyl or lower acyl.

3. The method of claim 1 or 2, wherein A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups.

4. The method of claim 3, wherein A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, A^9 is selected from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

5. The method of any one of claims 1 to 4, wherein A^{12} is selected from lower alkyl, lower alkene and lower alkyne.

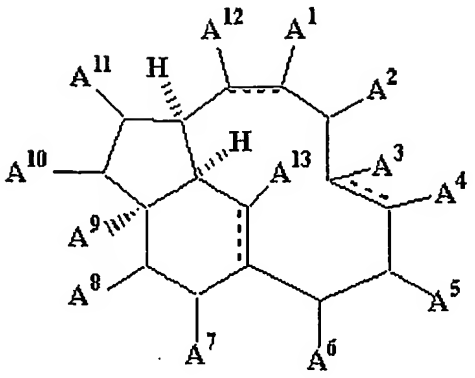
6. The method of claim 5, wherein A^{12} is selected from methyl and CH_2.

7. The method of any one of claims 1 to 6, wherein at least two of said A^1 to A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

8. The method of any one of claims 2 to 7, wherein R is methyl or ethyl.

9. The method of claim 1, wherein the compound is of the formula:

(2)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, lower alkyl, lower alkene, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups;

and pharmaceutically/veterinary-acceptable salts thereof.

10. The method of claim 9, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, O, and OR, and R in the group OR is a lower alkyl or lower acyl.

11. The method of claim 9 or 10, wherein A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy and lower alcohol groups.

12. The method of claim 11, wherein A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, A^9 is selected

from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

13. The method of any one of claims 9 to 12, wherein A^{12} is selected from lower alkyl, lower alkene and lower alkyne.

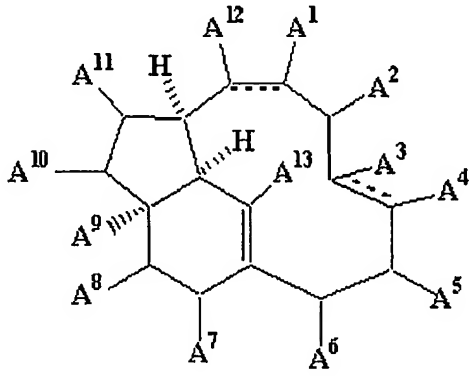
14. The method of claim 13, wherein A^{12} is selected from methyl and CH_2.

15. The method of any one of claims 9 to 14, wherein at least two of said A^1 to A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

16. The method of any one of claims 10 to 15, wherein R is methyl or ethyl.

17. The method of claim 1, wherein the compound is of the formula:

(3)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, acetoxy,

propionyloxy and butyryloxy groups and methanol, ethanol, propanol and butanol groups;
and pharmaceutically/veterinary-acceptable salts thereof.

18. The method of claim 17, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, O, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, acetoxy, propionyloxy and butyryloxy groups, and methanol, ethanol, propanol and butanol groups.

19. The method of claim 17 or 18, wherein A^4, A^9 and A^{13} are selected, independently, from methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, acetoxy, propionyloxy and butyryloxy groups, and methanol, ethanol, propanol and butanol.

20. The method of any one of claims 17 to 19, wherein A^{12} is selected from methyl, ethyl, propyl, butyl, methene, ethene and propene groups.

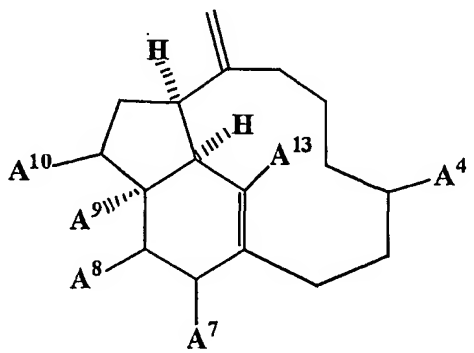
21. The method of claim 20, wherein A^{12} is selected from methyl and CH_2.

22. The method of any one of claims 17 to 21, wherein at least two of said A^1 to A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

23. The method of claim 22, wherein R is methyl or ethyl.

24. The method of claim 1, wherein the compound is of the formula:

(4)

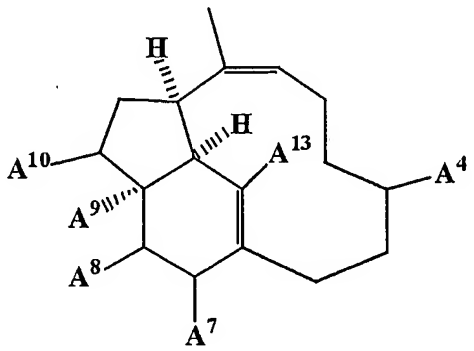


wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined in claim 1;
and pharmaceutically/veterinary-acceptable salts thereof.

25. The method of claim 1, wherein the compound is of the formula:

(5)



wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined in claim 1;
and pharmaceutically/veterinary-acceptable salts thereof.

26. The method of claim 24 or 25, wherein A^4, A^7, A^8 and A^{10} are selected, independently, from H, OH, O, SH, NH_2 and OR, and R in the group OR is a lower alkyl or lower acyl.

27. The method of any one of claims 24 to 26, wherein A^9 and A^{13} are selected, independently, from lower alkyl, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups.

28. The method of claim 27, wherein A^9 and A^{13} are selected, independently, from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

29. The method of any one of claims 24 to 28, wherein at least two of said A^4, A^7, A^8, A^9, A^{10} and A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

30. The method of any one of claims 26 to 29, wherein R is methyl or ethyl.

31. The method of claim 1, wherein the compound is selected from;
1(15),8(19)-Trinervitadiene-3α,5α,18-triol,
1(15),8(19)-Trinervitadiene-3α,5α-diol,
1(15),8(19)-Trinervitadiene-3α,5α,18-triol 5-acetate,
1(15),8(9)-Trinervitadiene-2β,3α-diol, and
1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate.

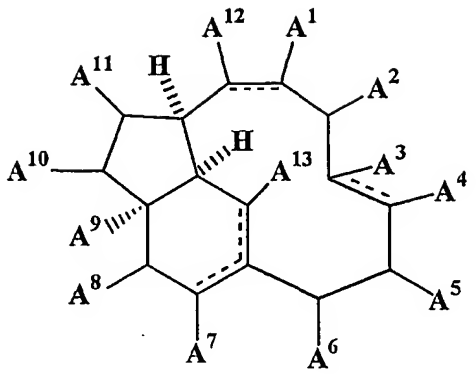
32. The method of any one of claims 1 to 31, wherein the compound or pharmaceutically/veterinary-acceptable salt thereof is administered to said subject in an amount in the range of 1 to 100 mg/kg.

33. The method of any one of claims 1 to 32, wherein the antimicrobial infection or disease is selected from bacterial infections of wounds, lung infections, skin infections and systemic bacterial infections.

34. A pharmaceutical and/or veterinary formulation for treating a microbial infection or disease in a subject, said formulation comprising a compound or pharmaceutical/veterinary-acceptable salt thereof as defined in any one of claims 1 to 31 in admixture witha suitable pharmaceutically/veterinary-acceptable excipient.

35. A method for disinfecting a surface, said method comprising applying to said surface an amount of a compound of the formula:

(1)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

(i) substituents A^1 to A^{13} are selected, independently, from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy, or

(ii) any one or more of substituent pairs A^1 and A^2, A^1 and A^3, A^2 and A^3, A^2 and A^4, A^3 and A^4, A^3 and A^5, A^4 and A^5, A^4 and A^6, A^5 and A^6, A^6 and A^7, A^7 and A^8, A^7 and A^9, A^8 and A^9, A^8 and A^{10}, A^9 and A^{10}, A^9 and A^{11}, A^{10} and A^{11}, A^{11} and A^{12}, A^1 and A^{12}, and A^2 and A^{12} form a substituted or unsubstituted heterocyclic group, wherein any substituents, including A^{13}, not forming a substituted or unsubstituted heterocyclic ring, are selected independently from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy;

with the provisos that,

only one of the bonds between C1 and C2, and C1 and C15, may be a double bond or epoxide,

when the bond between C1 and C2 is a double bond or epoxide, A^7 is bound to C2 by a single bond,

when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,
when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and
when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;
and salts thereof.

36. The method of claim 35, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, O, SH, NH_2 and OR, and R in the group OR is a lower alkyl or lower acyl.

37. The method of claim 35 or 36, wherein A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, and lower alcohol groups.

38. The method of claim 37, wherein A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, A^9 is selected from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

39. The method of any one of claims 35 to 38, wherein A^{12} is selected from lower alkyl, lower alkene and lower alkyne.

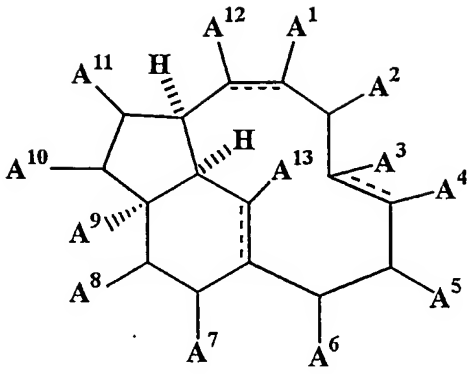
40. The method of claim 39, wherein A^{12} is selected from methyl and CH_2.

41. The method of any one of claims 35 to 40, wherein at least two of said A^1 to A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

42. The method of any one of claims 36 to 41, wherein R is methyl or ethyl.

43. The method of claim 35, wherein the compound is of the formula:

(2)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, lower alkyl, lower alkene, lower alkoxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, and lower alcohol groups; and salts thereof.

44. The method of claim 43, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, and OR, and R in the group OR is a lower alkyl or lower acyl.

45. The method of claim 43 or 44, wherein A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups.

46. The method of claim 45, wherein A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, A^9 is selected from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

47. The method of any one of claims 43 to 46, wherein A^{12} is selected from lower alkyl, lower alkene and lower alkyne.

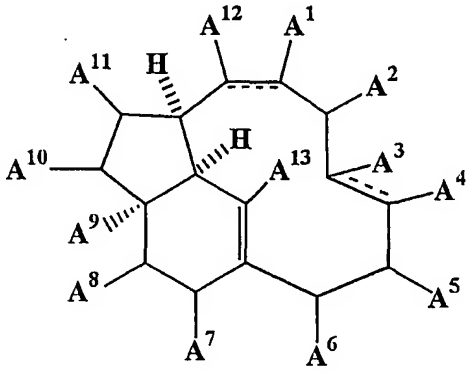
48. The method of claim 47, wherein A^{12} is selected from methyl and CH_2.

49. The method of any one of claims 43 to 48, wherein at least two of said A^1 to A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

50. The method of any one of claims 43 to 49, wherein R is methyl or ethyl.

51. The method of claim 35, wherein the compound is of the formula:

(3)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, acetoxy, propionyloxy and butyryloxy groups, methanol, ethanol, propanol and butanol groups, OCH and $OCCH_3$;
and salts thereof.

52. The method of claim 51, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate,

propanoate and butanoate groups, acetoxy, propionyloxy and butyryloxy groups, and methanol, ethanol, propanol and butanol groups.

53. The method of claim 51 or 52, wherein A^4, A^9 and A^{13} are selected, independently, from methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, methanol, ethanol, propanol and butanol.

54. The method of any one of claims 51 to 53, wherein A^{12} is selected from methyl, ethyl, propyl, butyl, methene, ethene and propene groups.

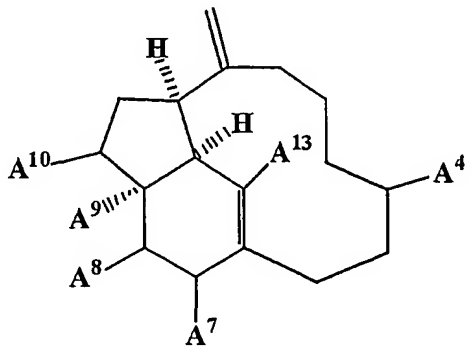
55. The method of claim 54, wherein A^{12} is selected from CH_2 and CH.

56. The method of any one of claims 51 to 55, wherein at least two of said A^1 to A^{12} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

57. The method of claim 56, wherein R is methyl or ethyl.

58. The method of claim 35, wherein the compound is of the formula:

(4)

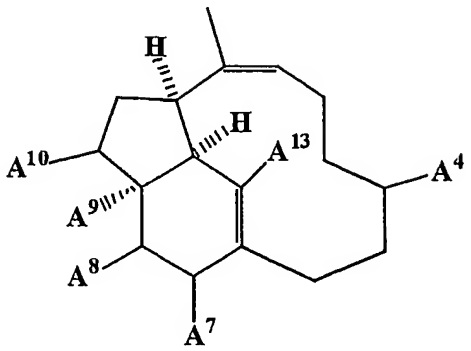


wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined in claim 35; and salts thereof.

59. The method of claim 35, wherein the compound is of the formula:

(5)



wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined in claim 35; and salts thereof.

60. The method of claim 58 or 59, wherein A^4, A^7, A^0 and A^9 are selected, independently, from H, OH, O, SH, NH_2 and OR, and R in the group OR is a lower alkyl or lower acyl.

61. The method of any one of claims 58 to 60, wherein A^9 and $A^{13}$are selected, independently, from lower alkyl, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy, and lower alcohol groups.

62. The method of claim 61, wherein A^9 is selected from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

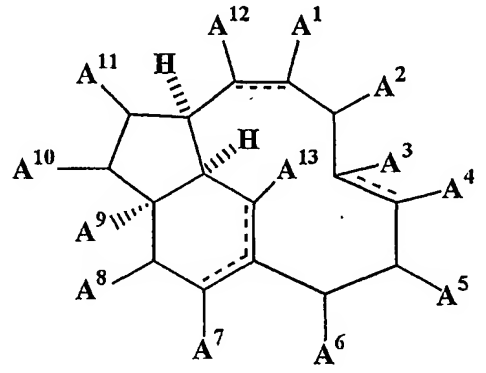
63. The method of any one of claims 58 to 62, wherein at least two of said A^4, A^7, A^8, A^9, A^{10} and A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

64. The method of any one of claims 58 to 63, wherein R is methyl or ethyl.

65. The method of claim 35, wherein the compound is selected from;
1(15),8(19)-Trinervitadiene-3α,5α,18-triol,
1(15),8(19)-Trinervitadiene-3α,5α-diol,
1(15),8(19)-Trinervitadiene-3α,5α,18-triol 5-acetate,
1(15),8(9)-Trinervitadiene-2β,3α-diol, and
1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate.

66. An antimicrobial compound of the formula:

(1)



wherein;

---- denotes a single or double bond or an epoxide bond, and

(i) substituents A^1 to A^{13} are selected, independently, from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy, or

(ii) any one or more of substituent pairs A^1 and A^2, A^1 and A^3, A^2 and A^3, A^2 and A^4, A^3 and A^4, A^3 and A^5, A^4 and A^5, A^4 and A^6, A^5 and A^6, A^6 and A^7, A^7 and A^8, A^7 and A^9, A^8 and A^9, A^8 and A^{10}, A^9 and A^{10}, A^9 and A^{11}, A^{10} and A^{11}, A^{11} and A^{12}, A^1 and A^{12}, and A^2 and A^{12} form a substituted or unsubstituted heterocyclic group, wherein any substituents, including A^{13}, not forming a substituted or unsubstituted heterocyclic ring, are selected independently from H, OH, O, SH, NH_2, lower alkyl, lower alkene, lower alkyne, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone

groups, lower ester groups, lower acyloxy groups, lower alcohol groups, lower alkylthio, lower alkylamino, lower alkysulfonyl, lower alkysulfinyl and lower alkylsulfonyloxy;

with the provisos that,

only one of the bonds between C1 and C2, and C1 and C15, may be a double bond or epoxide,

when the bond between C1 and C2 is a double bond or epoxide, A^7 is bound to C2 by a single bond,

when the bond between C1 and C15 is a double bond or epoxide, A^{13} is bound to C15 by a single bond,

when the bond between C8 and C9 is a double bond or epoxide, A^1 and A^{12} are bound to C9 and C8 respectively by a single bond, and

when the bond between C11 and C12 is a double bond or epoxide, A^3 and A^4 are bound to C11 and C12 respectively by a single bond;

and salts thereof, with the further proviso that said compound is not 1(15),8(9)-Trinervitadiene-2β,3α-diol.

67. The compound of claim 66, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, O, SH, NH_2 and OR, and R in the group OR is a lower alkyl or lower acyl.

68. The compound of claim 66 or 67, wherein A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups.

69. The compound of claim 68, wherein A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, A^9 is selected from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

70. The compound of any one of claims 66 to 69, wherein A^{12} is selected from lower alkyl, lower alkene and lower alkyne.

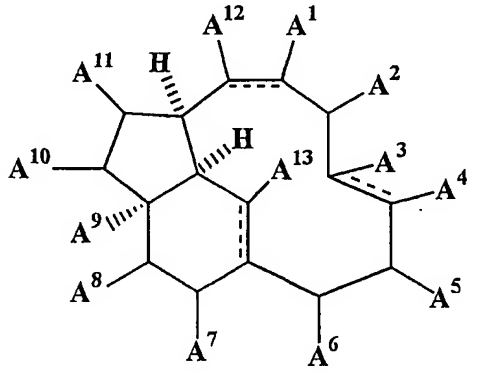
71. The compound of claim 70, wherein A^{12} is selected from methyl and CH_2.

72. The compound of any one of claims 66 to 71, wherein at least two of said A^1 to A^{12} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

73. The compound of any one of claims 67 to 72, wherein R is methyl or ethyl.

74. The compound of claim 66, wherein the compound is of the formula:

(2)



wherein;

substituents A^1 to A^{13} are selected, independently, from H, OH, O, lower alkyl, lower alkene, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups, and lower alcohol groups;
and salts thereof.

75. The compound of claim 74, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, and OR, and R in the group OR is a lower alkyl or lower acyl.

76. The compound of claim 74 or 75, wherein A^4, A^9 and A^{13} are selected, independently, from lower alkyl, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups.

77. The compound of claim 76, wherein A^4 and A^{13} are selected, independently, from methyl, methanoate and methanol groups, A^9 is selected from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

78. The compound of any one of claims 74 to 77, wherein A^{12} is selected from lower alkyl, lower alkene and lower alkyne.

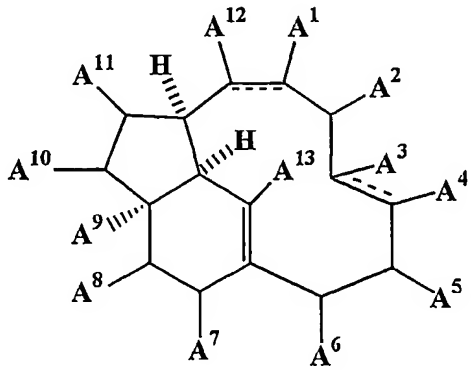
79. The compound of claim 78, wherein A^{12} is selected from methyl and CH_2.

80. The compound of any one of claims 74 to 79, wherein at least two of said A^1 to A^{12} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

81. The compound of any one of claims 75 to 84, wherein R is methyl or ethyl.

82. The compound of claim 66, wherein the compound is of the formula:

(3)



wherein;

---- denotes a single or double bond or an epoxidised bond, and

substituents A^1 to A^{13} are selected, independently, from H, OH, O, methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, acetoxy, propionyloxy and butyryloxy groups, and methanol, ethanol, propanol and butanol groups;
and salts thereof.

83. The compound of claim 82, wherein A^1, A^2, A^3, A^5, A^6, A^7, A^8, A^{10} and A^{11} are selected, independently, from H, OH, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, methanol, ethanol, propanol and butanol groups.

84. The compound of claim 82 or 83, wherein A^4, A^9 and A^{13} are selected, independently, from methyl, ethyl, propyl, butyl, methene, ethene and propene groups, methanal, ethanal, propanal, butanal, methanone, ethanone and propanone groups, methanoate, ethanoate, propanoate and butanoate groups, methanol, ethanol, propanol and butanol.

85. The compound of any one of claims 82 to 84, wherein A^{12} is selected from methyl, ethyl, propyl, butyl, methene, ethene and propene groups.

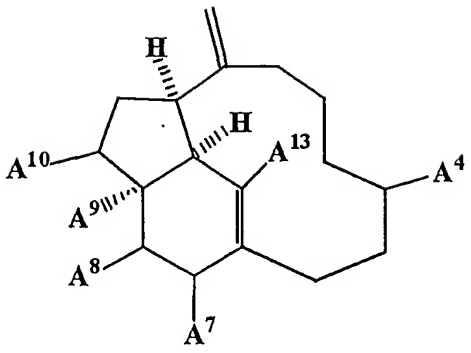
86. The compound of claim 89, wherein A^{12} is selected from methyl and CH_2.

87. The compound of any one of claims 82 to 86, wherein at least two of said A^1 to A^{12} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

88. The compound of claim 87, wherein R is methyl or ethyl.

89. The compound of claim 66, wherein the compound is of the formula:

(4)



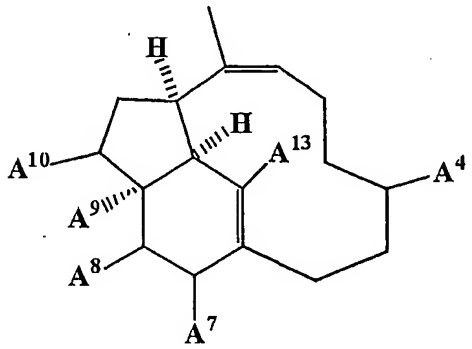
wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined in claim 66;

and salts thereof.

90. The compound of claim 66, wherein the compound is of the formula:

(5)



wherein;

substituents A^4, A^7, A^8, A^9, A^{10} and A^{13} are as defined in claim 66;

and salts thereof.

91. The compound of claim 89 or 90, wherein A^4, A^7, A^8 and A^{10} are selected, independently, from H, OH, O, SH, NH_2 and OR, and R in the group OR is a lower alkyl or lower acyl.

92. The compound of any one of claims 89 to 91, wherein A^9 and A^{13} are selected, independently, from lower alkyl, lower alkoxy, lower carboxy, lower aldehyde groups, lower ketone groups, lower ester groups, lower acyloxy groups and lower alcohol groups.

93. The compound of claim 96, wherein A^9 and A^{13} are selected, independently, from methanol and CH_2OR groups, and R in the group OR is a lower alkyl or lower acyl.

94. The compound of any one of claims 89 to 93, wherein at least two of said A^4, A^7, A^8, A^9, A^{10} and A^{13} consist or comprise OH or OR groups, and R in the group OR is a lower alkyl.

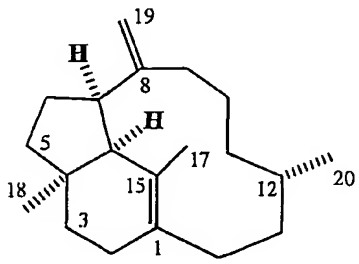
95. The compound of any one of claims 89 to 94, wherein R is methyl or ethyl.

96. The compound of claim 66, wherein the compound is selected from;
1(15),8(19)-Trinervitadiene-3α,5α,18-triol,
1(15),8(19)-Trinervitadiene-3α,5α-diol,
1(15),8(19)-Trinervitadiene-3α,5α,18-triol 5-acetate, and
1(15),8(19)-Trinervitadiene-3α,5α,18-triol 3,5,18-triacetate.

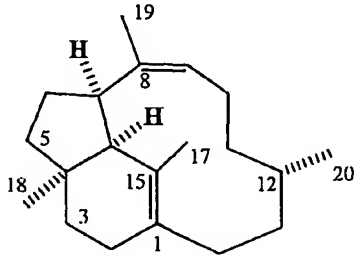
97. The compound of any one of claims 66 to 96, wherein the compound is in a substantially purified form.

98. An antimicrobial trinervitadiene compound in a substantially purified form, said compound being obtainable from a termite of the genus Nasutitermes.

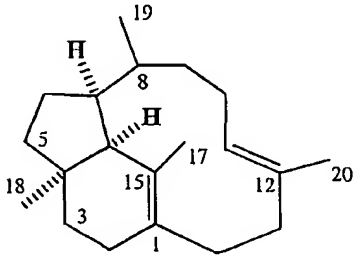
Figure 1: Trinervitadiene ring system and naturally occurring unsaturated forms:



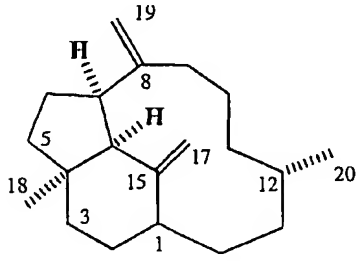
11: 1(15),8(19)-Trinervitadiene



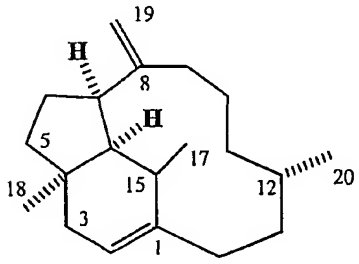
12: 1(15),8(9)-Trinervitadiene



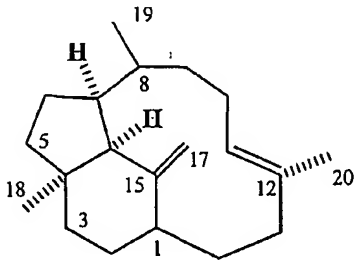
13: 1(15),11(12)-Trinervitadiene



14:.. 8(19),15(17))-Trinervitadiene



15: 1(2),8(19))-Trinervitadiene



16: 11(12),15(17))-Trinervitadiene

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00589

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07C 13/573; A61K 31/015, 31/045, 31/047, 31/185; A61L 2/18; A61P 31/04, 31/06, 31/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: Sub structures based upon trinervitane skeleton. Keywords: trinervitane, trinervitene.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Kato, T et al, Helv. Chim. Acta. (2001), 84(1), 47-68. Cyclization of polyenes, Part 60. Construction of trinervitane and kempane skeletons based on biological routes". The whole document.	66-72, 74-80, 82-87, 89-92, 94, 97-98
X	Valterova, I *et al*, Collect Czech Chem Commun (1991), 56 (12), 2969-77. "Defensive substances from the frontal gland secretion of *Nasutitermes nigriceps* termite soldiers". Structures I to XIII	66-72, 74-80, 82-87, 89-92, 94, 97-98

Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of Mailing of the International Search Report
25 July 2001	22 August 2001
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer D.A. Lally D.A. LALLY Telephone No : (02) 6283 2533

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00589

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chuah, C.H. *et al*, Biochem Syst Ecol (1991), 19 (1), 35-46. "Intra- and interspecific variations in the defense secretions of the Malaysian termite *Hospitalitermes*". Compounds 12 to 26.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Chuah, C.H. *et al*, J. Chem Ecol. (1989), 15 (2), 549-63. "Interspecific variation in defense secretions of Malaysian termites from the genus *Nasutitermes*" (Isoptera: Nasutitermitinae). Compounds VII, VIII, IX, XI, XII, XVII, XIII, XVIII, XIX, XX, XXI, XXII.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Roisin, Y. *et al*, Biochem Syst Ecol (1987), 15 (2), 253-61. "Soldier diterpene patterns in relation with aggressive behaviour spatial distribution and reproduction of colonies in *Nasutitermes princeps*". Table 1.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Valterova, I *et al*, Collect Czech Chem Commun (1986), 51 (12), 2884-95. "Constituents of frontal gland secretion of Peruvian termites *Nasutitermes ephratae*". Structures I, Ia, II, III, IV, V, VII, XI, XII, XIII.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Chuah, C.H. *et al*, J Chem Ecol (1986), 12 (3), 701-12. "Soldier defense secretions of the genus *Hospitalitermes* in peninsular Malaysia". Structures X to XX.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Valterovna, I *et al*, Collect Czech Chem Commun (1984), 49 (9), 2024-39. "Minor diterpene components of the defense secretion from the frontal gland of soldiers of the species *Nasutitermes costalis* (Holmgren)". Structures I to IVf, IX to XV.	66 to 71, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Braekman, J.C. *et al*, Bull Soc Chim Belg (1984), 93 (4), 291-7. "New trinervitane diterpenes from Neo-Guinean *Nasutitermes* sp". Structures 1 to 9.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00589

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Braekman, J.C. *et al*, Tetrahedron (1983), 39 (24), 4237-4241. "Chemical composition of the frontal gland secretion from soldiers of *Nasutitermes lujae* (Termitidae, Nasutitermitinae). Structures 5 to 9, 11 to 14.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Braekman, J.C. *et al*, Bull. Soc. Chim. Belg. (1983), 92 (2), 111-14. "3 alpha-hydroxy-8-beta-trinervita-1,11-diene: a novel diterpene from two *Trinervitermes* species". Structures 2, 6, 7, 8.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Baker, R. *et al*, Tetrahedron (1982), 38 (13), 1899-910. "Soldier defense secretions of the South American termites *Cortinaritermes silvestri*, Nasutitermes sp N.D, and Nasutitermes kemneri".	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Dupont, A. et al, Bull. Soc. Chim. Belg. (1981), 90 (5), 485-99. "Chemical composition of the frontal gland secretions from Neo Guinean nasute termite soldiers". Structures 5 to 9, 11 to 15, 17 to 27.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Prestwich, Glenn D. *et al*, Tetrahedron Lett (1981), 22 (17), 1563-6. "New tricyclic diterpene propionate esters from a termite soldier defense secretion". Structures 1 to 3, 5 to 7.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Prestwich, Glenn D. *et al*, J Chem Ecol (1981), 7 (1), 147-57. "Soldier defense secretions of *Trinervitermes bettonianus* (Isoptera, Nasutitermitinae): chemical variation in allopatric populations". Structures 7 to 10.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Prestwich, Glenn D. *et al*, Insect Biochem (1979), 9 (6), 563-7. "Defense secretion of the black termite, *Grallatotermes africanus* (Termitidae, Nasutitermitinae)". Structures 8 to 11.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00589

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Vrkoc J. *et al*, Collect. Czech. Chem. Commun. (1978), 43 (9), 2478-85. "Structure of 2α, 3α -dihydroxy- and 2α, 3β -dihydroxy-1(15), 8(19)-trinervitadienes from *Nasutitermes castalis* (Holmgren)". See whole document.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Prestwich, G.D., Experentia. (1978), 34 (6), 682-4. "Isotrinervi-2β-ol. Structural isomers in the defense secretions of allopatric populations of the termite *Trinervitermes gratiosus*". See whole document.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98
X	Prestwich, Glenn D., *et al*, J. Am. Chem. Soc. (1976), 98 (19), 6062-4. "Nasute termite soldier frontal gland secretions. 2. Structures of trinervitine congeners from *Trinervitermes* soldiers". See whole document.	66 to 72, 74 to 80, 82 to 87, 89 to 92, 94, 97, 98